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Additional inventors are being named on the none separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

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A simultaneous multi-column liquid chromatograph for direct sampling of an array of liquid samples.

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This invention relates to making simultaneous separation-based chemical analyses of liquid samples. Specifically, the liquids are contained in a rectangular array of recesses, here called wells, in a planar arrangement in a holder, here called a microtiter plate. Separation-based chemical analysis involves the taking of an aliquot of a liquid sample. A device approaches the surface of the microtiter plate, makes a connection with the liquid in several of the wells, and withdraws the aliquots. Each aliquot of liquid is further processed by being carried in a flow of a carrier liquid, here called a mobile phase, in a further step that differentially separates different chemical components in the sample. Common examples of this separation are liquid chromatography and electrophoresis.

In the pharmaceutical industry, such arrays of liquid samples are routinely used, and subjected to many kinds of chemical analysis, including separation-based analysis, such as high performance liquid chromatography (HPLC). These arrays are processed in microtiter plates, are important in the fields of drug discovery and drug development.

It is understood that devices performing separation-based chemical analyses may additionally or even principally perform other chemical functions, such as reaction, filtration, purification, fractionation, or measurement of other properties, in addition to chemical analysis.

It is understood that when this invention is used for separation-based chemical analysis, each sample may also undergo subsequent further chemical analysis in a separate instrument, such as a mass spectrometer.

The fundamental idea is to make a series of devices, capable of being arranged in an array, small enough to be mounted over a microtiter plate of standard size, where each

device can communicate with a well of the microtiter plate simultaneously, and that in one or more cycles of operation, all of the samples in the wells in the microtiter place can be chemically analyzed.

Inventive features.

Others have previously made a combination of chemical analyzers compact enough to process multiple samples. For instance Biotage, and the DNA analyzers. Those devices are compact, do multiple simultaneous samples, have a chromatographic separation (by capillary electrophoresis) and a detection step (laser induced fluorescence).

Some notable features of this invention, compared to some of the existing techniques are:

1. High performance liquid chromatography is used, which requires high pressures (> 3000 psi)
2. The several elements are connected directly together, eliminating interconnecting tubing, which can reduce performance.
3. Is compact (most equipment can be installed in a single small box)
4. Several chemical analysis devices are mounted in a sub-unit, for instance, in an array of eight. Multiple sub-units can be ganged together to form larger arrays (this places limits on the sizes of individual devices and on sizes of the sub-units.
5. The injection device itself is inventive.

The Injector.

In traditional chromatographic equipment, especially high-pressure types, the sample is injected using a sampling valve, involving 1. Transferring a flow of the sample to a tubular volume (or sample loop) 2. A valve actuator changes the state of the sampling valve, and 3 the tubular volume is switched into the flow path between the source of mobile phase and the separating device, or column.

The sampling valve is too large to fit over a well of a microtiter plate, so that devices conduct or transfer some of the sample to be analyzed to the specialized valve located some distance away. A moving syringe pump samples and delivers from the well to the inlet of the sampling valve. This involves other devices contacting the liquid, or long tubes carrying it, so that the flow of sample is spread out – and the chromatographic separation is of lower quality.

In this invention, the functions of valve actuator, sampling valve, syringe pump, and transfer device are all integrated into a single injector mounted at the end of the chromatographic column. The combination of the injector, chromatographic column, detector and auxiliary tubing and conduits needed to support the operation, all fit into a 9 mm spacing, so that eight combinations can be mounted side by side, and simultaneously service 8 chemical samples in a row of the microtiter plate.

Advantages of the invention compared to traditional HPLC.

In multiplexed analysis, it is common to use a common method for each channel: same column, flow gradient, sample injection and so forth. The samples analyzed are expected to be somewhat similar.

As such, it is an advantage of this invention that multiple injections are performed in the same step, rather than having to operate an injection mechanism successively. The advantage is in time and simplicity (cost and reliability).

The flow and profiles of the columns are the same or very similar. It is an advantage of this invention that it takes advantage of this similarity to control flows to each column simply by spitting the flow into several nearly equal flows, relying on the similarity of the column flow resistances to give nearly equal flows.

Since the flows will not be identical, software adjustments are used to compensate for the small differences.

In conventional HPLC, variations in flow, viscosity, and pressure make it difficult to inject a known volume. Therefore conventional injectors fill a known volume with the sample liquid at low pressure, and then insert the volume into a high-pressure flow path.

In a multiplexed operation, the physical conditions of flow etc., are much more controlled. Therefore it is possible to use a known volume (created by a device like a syringe pump) to set the total volume, and use the uniformity of the columns to allocate this volume among the injection volumes of each injector.

In a conventional injection valve, a electrical solenoid or is commonly used to switch the state of the injection valve. But since multiplexed chromatography used a known physical environment (taking samples from a microtiter plate), The need for an electrical solenoid can be avoided, in one implementation. In this invention, the valve introducing the sample is opened by pressing the tip of the injection needle against the bottom of the sample container (usually a microtiter plate).

Object of this invention.

1. Use of integrated LC to simultaneously inject and chromatographically analyze multiple samples simultaneously from a microtiter plate.

2. The use of a combination of a poppet valve, hollow needle, and reversible pump as an injection device for a chromatographic system. Here, a "poppet valve" is a valve with a pair of sealing surfaces that can be opened or closed by motion of a rigid part connected to one of the sealing surfaces. For instance, the "ball-needle" valve described here, has a spherical metal sealing surface, rigidly attached to a rigid hollow needle. When the needle is pressurized, the spherical surface separates from the mating sealing surface, which has a conical shape.

3. A method to run multiple versions of the same chromatographic measurement, with a single source of solvent, with:

1) Solvent splitting among the N columns, from a single pressurized source.

2) Substantial flow equality by using identical columns as flow restrictors,

3) Adjusting for variations in flow, due to differential plugging or aging of the columns,

By adjusting the time base of the chromatographic data of each channel,

Or by adjusting the time base of the integration and computer program that analyzes the data.

4. – the method of #3 where there are several independent sources of flow.

5. A compact HPLC, consisting of a rigidly mounted combination of an injection device, filter, column and detector, suitable for being positioned near a microtiter place, and aspirating samples directly from it..

6. A compact HPLC, as in #4, of such size that several such HPLC units can be mounted together, suitable for use with a microtiter plate.

7. A compact HPLC as in #4, or an array of compact HPLCs, as in #5, which can be moved into contact with a microtiter plate, or an array of containers with liquids, and then be made to take a sample or samples, and perform chromatographic separation.

Figures.

Figure 1 shows a schematic diagram of the prior art.

Figure 2 shows the basic injector attached to a column and hollow needle.

Figure 3 shows an example of interior structure of the basic injector.

Figure 4 shows a functional device incorporating multiple basic injectors, suitable for multiple sample aspirations from a microtiter plate and chromatographic separation and detection.

(The is no Figure 5)

Figure 6 shoes a functional device incorporating several rows of basic injectors.

Figure 7 shows an alternative detail of the hollow needle, so that pressing against a different surface can activate the poppet valve.

Figure 8, shows the functional device of Figure 6, used with an alternative location form device washing and rinsing.

Figure 9 shoes a two-injector device, made from discrete tubing lengths and Tee connectors.

Figure 10 shows the integration of the sample tubes into a sample tube block and the resulting simplified design, along with the dual flow paths to the sample tubes, which permit greater control over sample volume.

Figure 11 shows a design where each injector has its own source of flow, but shares a common syringe pump for aspiration.

Figure 1 shows a multiple analysis system for HPLC (high performance liquid chromatography) capable of analyzing samples from a microtiter plate, 20, according to the prior art. A syringe, 30, is used to take samples and deliver them to injection valves, 6, at a first valve position, 26, and a second valve position, 27. The syringe, 30, comprises a syringe needle, 31, a syringe barrel, 33, a liquid-tight plunger, 32, and a plunger moving means, 34, by which the liquid-tight plunger, 32, can be moved within the syringe barrel, 33, causing liquid to be sucked into the syringe, 30, or to be expelled.

A microplate, 20, has a series of wells, 21, for the purpose of containing liquid samples, 22. An automated mechanism, not shown, moves the syringe to a microplate position, 23, so that the syringe needle, 31, comes in contact with a liquid sample, 22. The syringe system then aspirates a certain volume of the liquid sample, 22.

There are multiple valves, 3, suited to injecting liquid samples into HPLC analysis systems. Each valve, 3, comprises a sampling loop, 4, a valve exit tube, 12, and a port, 6, suitable for receiving a syringe needle.

The syringe, 30, which contains a certain amount of sample, is moved to a first valve position, 26, where the syringe needle, 31, is engaged in a port, 6, so that a liquid sample can be injected into a sampling loop, 4, with any excess, passing through the loop, 4 and out a vent exit tube, 12.

The plunger moving means, 34, causes the plunger, 32, to expel the liquid into the loop, 4.

For each valve, 3, there is a pump, 1, and a delivery tube, 2, which delivers a liquid flow to the valve, 3. From each valve, 3, a transfer tube, 5, a chromatographic column, 7, a column exit tube, 8, a detector, 9, and a detector exit tube, 12, carries a liquid flow out of the valve, 3.

When a valve, 3, is caused to change its state, the loop, 4, is inserted into the flow path between delivery tube 2, and transfer tube, 5, so that the contents of the loop, 4, including liquid sample injected by the syringe, 30, are swept through the transfer tube, 5, to the chromatographic column, 7.

The constituents of the liquid sample travel through the column at different rates, as is well known. The effluent from the column, 7, flows through column exit tube, 8, to a detector, 9. The detector, 9, is configured to measure the concentration of chemical samples in the liquid, and to produce a continuous record of the varying level of concentration. Such record indicates three things: a variation in the measurement indicates the presence of various chemicals in the sample; the times of the variations indicate the identities of said chemicals; and the intensities of the variations indicate the concentration of the chemicals.

In a multi-HPLC analyzer, the syringe, 30, delivers samples consecutively from the microplate to several chromatographic systems. For instance, the syringe, 30, after delivering a sample from first microplate position, 23, to first valve position, 26, can deliver another sample from second microplate position, 24, to a second valve position,

27. In a multi-HPLC analyzer, there is a multiplicity of pumps, 1, delivery tubes, 2, valves, 3, transfer tubes 5, columns 7 and detectors, 9.

It is clear to those skilled in the art, that multiple syringes can be used instead of a single syringe, and that multiple syringes can operate simultaneously or consecutively. It is clear that one or more syringes can make injections from either one microplate position, or in a sequence of operations, from more than one position, including especially, all of the positions in the microplate. Those skilled in the art will also find it obvious for the syringe or syringes to undergo a cleaning step between injections, consisting of rinsing the syringe with one or more solvents. It is clear that the chromatographic process begins when valve, 3, rotates the loop, 4, into the main flow stream, so that a syringe, 30, can consecutively fill several loops, 4, followed by simultaneous action by each valve, 6, so that the chromatographic processes take place simultaneously.

Figure 2 shows a liquid chromatograph that incorporates the injector according to this invention.

A reservoir, 40, contains a liquid, 41, used as the mobile phase for the liquid chromatographic process. A pump, 42, withdraws said liquid, 41, through a liquid supply tube, 43, which is connected both to the reservoir, 40, and to the pump, 42.

The pump, 42, also delivers the liquid, 41, to the injector, 45, though a tube, 44, which is connected both to said pump, 42, and to said injector, 45. The liquid, 41, flows through the injector, 45, and then to a chromatographic column, 46, though an adaptor fitting, 47.

From the chromatographic column, 46, the liquid, 41, flows into a detector, 48, through a detector adaptor fitting, 49. By processes well known to those skilled in the art, continuous chemical measurements are made by the detector on any chemicals contained in the detector, 48, by the flow of liquid, 41. An electronic readout of these measurements is carried to a computer, 50, though a cable, 51. Liquid flows out of the detector, 48, though vent tube, 58.

The injector, 45, also contains a hollow needle, 52, and said injector, 45, is attached to an actuator, 53. The actuator, 53, can move the injector, 45, to a container, 54, for the purpose of taking a sample of a liquid chemical, 55, contained therein. The actuator moves the injector, 45, over the container, 54, and lowers the injector, 45, so that the needle, 52, enters the container, 54, and presses against the container bottom, 56.

This causes the needle, 52, to move upward within the injector, 45, such movement opening a passage between the needle, 52, and the tube, 44.

In operation, a sample of the liquid chemical, 55, is introduced onto the column, 46, in several steps.

a) The liquid pumping system, 42, interrupts the flow of liquid to the injector, and waits for a time sufficient for the liquid pressure within the injector, 45, to drop below a low value convenient for introducing a sample. A typical low-pressure value is 500 psi.

b) The actuator, 53, moves the injector, 45, to a position over the container, 54, and lowers the injector, 45, so that the needle, 52, presses against the container bottom, 56. This movement opens a passage from the chemical 55, through the needle, 52, through the injector, 45, to the tube, 44.

c) The pump, 42, aspirates a certain amount of liquid out of tube 44, into pump, 42. This in turn sucks liquid out of the sample container, 54, through the needle, 52, though the injector, 45, and into said tube 44. During this process, a negligible amount of liquid is also sucked out of column, 46 and column adaptor 47, but the amount of this liquid flow is much less than the flow from the container, 54, since the resistance to flow within the column, 46, is much higher than the resistance to flow within the needle, 52.

d) Once the desired amount of liquid sample from the chemical, 55, has entered the tube, 44, the actuator, 53, raises the injector, 45, from the container, 54, so that the needle, 52, no longer holds open a passage from the needle, 52, to the tube, 44.

e) The liquid pumping system, 42, resumes delivery of liquid through tube, 44, and successively into the injector, 45, column adaptor fitting, 47, column, 46, detector, 48, and vent tube, 58. This flow of liquid carries the sample deposited in tube 44 into the column, for the purpose of chromatographic separation and detection of the constituents of the liquid chemical, 55, as is well known to those skilled in the art.

f) When the chromatographic separation and detection of the sample is completed, the injector and needle are rinsed. The liquid pumping system, 42, interrupts the flow of liquid to the injector, 45, and the pressure drops. The actuator, 53, moves the injector, 45, to a rinsing container, 60, where the needle, 52, is pressed against the rinsing container bottom, 61, thereby opening a passage between the needle, 52, and the tube, 44. Now the liquid pumping system sends liquid through the tube 44, successively through the injector, 45 and needle 52. The amount of this flow is set to be sufficient to remove residues of the sample taken during the aspiration of sample from the liquid chemical, 55, so that subsequent samples will be substantially free of contamination from previous samples.

Figure 3 shows one embodiment of the injector, 45, which is mounted on a mechanical transporter, 53.

There is an upper liquid flow path, composed of the tube, 44, connected to an injector body, 45, with a tube fitting, 69. The tube, 44, is capable of sustaining the high pressures typical of HPLC, such as 350 atmospheres, and has sufficient internal volume to contain the volume of sample liquid used for chromatography, such as 10 microliters.

The flow path includes a flow passage, 70, within the injector body, 45. The flow passage, 70, is limited to a very small volume, with all parts well-swept by the liquid

flow, so that when liquid samples are transferred to the column through the flow passage, 70, there is no broadening of the chromatographic output due to dispersion of the sample while in the flow passage 70. The flow passage, 70, communicates with an adaptor fitting, 47. During operation, the tube, 44, conducts a solvent liquid into the injector body, 45. The solvent liquid flows through the flow passage, 70, and then through the adaptor fitting 47, and into the column.

A ball, 71, makes an intermittent seal against a valve seat, 72. The ball, 71, is rigidly attached to a hollow needle, 52. The ball, 71, is sufficiently round, smooth and concentric with respect to the hollow needle, 52, to serve as a high-pressure seal, in combination with the valve seat, 72.

The valve seat, 72, is designed to form a high-pressure seal with ball, 71. So it is either hard and very smooth or somewhat smooth and resilient, as is necessary in forming a high-pressure seal. The seal between the ball, 71, and valve seat, 72, is suitable for intermittent operations, with many thousands of cycles before replacement, and can seal against the high pressures typical in HPLC, such as 350 atmospheres.

The hollow needle, 52, has a bottom opening, 80, communicating with a top opening, 74. The hollow needle, 52, is sufficiently narrow near the bottom opening, so that the hollow needle can easily be positioned within a well in a microtiter plate. In operation, a mechanical transporter, 53, moves the injector, 45, against a microtiter plate, so that the hollow needle, 52, is positioned within a well of the microtiter place, and is pressed against the bottom of said well to open the seal of the ball, 71. The hollow needle, 52, is sufficiently rigid that it will not buckle when pressed against the bottom of the well.

The hollow needle, 52, is positioned so that it penetrates a central hole in the valve seat 72, and it is spring loaded by spring, 77, to pull the ball, 71, against the valve seat, 72, so that said ball, 71, and said valve seat, 72, form a liquid-tight seal.

In operation, the valve seat, 72 is mounted in the injector body, 45, and pressed against the injector body, 49, forming a seal. The two seals, between the ball, 71, and the valve seat, 72, and between the valve seat, 72 and the injector body, 45, prevent flow from the flow passage, 70 from entering the hollow needle, 52.

The combination of the ball, 71, hollow needle 52, valve seat, 72, and spring, 77, are mounted into the injector nut, 75. The valve seat, 72, is first mounted in or next to a back ferrule, 73, which is in turn pressed against the injector nut. 75. The injector nut, 75, is a threaded part, designed to be attached to the injector body, 49, for the purpose of supporting the ball, 71, valve seat 72, and hollow needle, 52. The back ferrule, 73, prevents torque being applied to the valve seat, 72, when it is being sealed against the injector body, 49.

The ball, 71, is positioned against the valve seat, 72, so that the hollow needle, 52, penetrates the valve seat, 72, the back ferrule, 73, and the injector nut, 75, and protrudes beyond the far edge of the injector nut, 75. The spring, 77 is mounted around the hollow needle, 52, from the far side, and is compressed between the injector nut, 75, and a collar, 78, and a crimp, 79. The combination of collar, 78 and crimp, 79, are attached to the hollow needle, 52, in such a way that the compressed spring, 77, applies a force to the hollow needle, 52, and thence to the ball, 71, which induces the ball, 71, to make a seal against the valve seat, 72. For instance, with a 2 mm diameter ball, a force of 2 pounds is sufficient to make a seal. The crimp, 79, may be attached to the hollow needle, 52, by brazing, crimping, gluing, or by fitting into a groove in the hollow needle, as an E-ring.

An O-ring, 76 is mounted around the hollow needle, 52, and between the injector nut, 75 and the back ferrule, 74. The O-ring, 76, seals the hollow needle, 52, to the injector nut, 75, and seals to the back ferrule, 73. The outside of the hollow needle, 52, is smooth so that it can form a sliding seal against the O-ring, 76. The O-ring, 76, need only withstand lower pressures, such as 20 atmospheres, since in normal operation, the high pressures used in HPLC are tuned off before the ball, 71, opens, and exposes the O-ring, 76, to the higher pressures that the ball, 71, is designed to seal against.

The top opening, 74, in the hollow needle, 52, is located below the seal between the valve seat, 72 and the ball, 71, but above the seal between the hollow needle, 52, and the back ferrule, 73.

The bottom opening, 80, allows sample to enter from a well, but the edge of the hollow needle, 52, around the bottom opening, must be robust enough to support pressure between the well bottom and the hollow needle, 52.

The space around the hollow needle, 52, below the ball, 71, and above O-ring, 75, needs to have minimal volume and be easily cleaned by rinsing, so that residual liquid from one sample may be prevented from substantially contaminating a subsequent sample.

The clearance between the hollow needle, 52, and injector nut, 76, needs to allow freely sliding relative motion, but narrow enough to prevent the tilt of the hollow needle sufficient to interfere with the hollow needle passing into a well in a microtiter plate.

When multiple injectors, 54, are installed together, the flow characteristics, especially in the hollow needles, 52, and the tubes, 44, must be substantially the same, so that when sample is aspirated, and the samples enter the tubes, the flows will be substantially the same in each injector, 54, and substantially the same amounts of samples will enter each tube.

The ball, 71, is preferentially kept small, such as 2 mm in diameter, so that lower forces are required from the spring, 77, to make a seal, and to minimize the volume of the flow passage, 70, which is typically less than 10 microliters. The tube, 44, should be narrow to minimize broadening of the sample distributions during chromatography, such as 0.1 to .5 mm diameter. The volume of the tube, 44, however, needs to be sufficiently large to contain the largest sample injection volume, such as 20 microliters. The above numbers have been found suitable for a certain size of chromatography column, 2 mm diameter

and 20 mm length, with a flow rate of 1 to 2 mL per minute. It will be appreciated by those skilled in the art that the above-mentioned dimensions should be scaled, when other chromatographic conditions are used, such as a chromatographic column of different diameter. For instance, with a 4.6 mm diameter chromatographic column, the volumetric dimensions should be about 10 times larger; with a 1 mm column, they should be about 10 times smaller.

Figure 4 shows the invention used for multi-channel chromatographic analysis of liquid chemical samples contained in a microtiter plate, 103, which has a pattern of 96 wells that are arranged in a rectangular array of 8 x 12 wells, based on a unit cell of 9 by 9 mm square. Other versions of microtiter plates have 384 wells in a 16 x 24 array on 4.5 mm centers, or 1536 wells, in 32 x 48 array, on 2.25 mm centers.

Several columns, 86, such as the four columns shown in Figure 4, are mounted on a series of injectors, 83. The injectors, 83, may be fashioned out of separate parts, and mounted together as shown in Figure 4, or machined in a common block. The injectors, 83, are rigidly mounted onto a mechanical transporter, 82. The spacing between the hollow needles, 84, which are incorporated into said injectors, is 9 mm, to match the spacing between the wells of the microtiter plate, 103.

The injectors, 83, can each supply liquid to, and receive liquid from, tubes, 92. These tubes, 92, all connect to a union fitting, 93, which then is connected to a supply tube, 94.

Pump Selection Valve.

During chromatography, the liquid chromatography pump, 96, through pump tube, 99, supplies flow at high pressure to the injectors, 86, and columns 83. The syringe pump, 100, and syringe control valve, 112, are generally not designed to withstand the pressures used in liquid chromatography, which can exceed 5000 psi.

Therefore a pump selection valve, 95, is interposed between the flow from the pump tube, 99, and the supply tube, 94. During chromatography, the pump selection valve, 95 is in the "pump" position, 97, permitting flow from the liquid chromatography pump, 96. When the syringe pump, 100, is in use, the pump selection valve, 95, connects to said syringe pump, 100, in place of the liquid chromatography pump, 96, by switching to the "aspirate" position, 98.

Syringe Control Valve.

The flow from the pump selection valve, 95, through the aspiration tube, 111, the syringe tube, 109, to the syringe pump, 100, goes through the syringe control valve, 112, when said syringe control valve is in the "fill" valve position, 113. Said valve, 112, in its "vent" valve position, 114, can divert this flow through the vent tube 116, to vent to waste, 117, by. Said valve can also, it its "empty" valve position, 115, can allow the syringe pump to empty to vent to waste, 117.

Syringe pump.

The syringe pump, 100, has a syringe plunger, 101, connected to a syringe driver, 102. The syringe driver, 102, can move the syringe plunger, 101, with respect to the syringe pump, 100, so that the syringe pump, 100, can either aspirate liquid when the syringe plunger, 101, is pulled out, or can deliver liquid, when the syringe plunger, 101, is pushed in.

Some types of syringe pumps are designed for high pressures. In this case, as is obvious to those skilled in the art, the pump selection valve, 95, can be replaced by a "Tee" connection between the two pump tubes, 99, and the supply tube, 94. Alternatively, one or more syringe pumps, 100, can be adapted to serve as both the liquid chromatography pump and the pump used to aspirate and deliver samples.

Aspiration.

In preparing for chromatographic injection, the mechanical transporter, 82, moves the injectors, 83, so that the hollow needles, 84, which are incorporated into said injectors, simultaneously press against the bottom of a series of wells, 104, in the microtiter plate, 103. Then, with the pump control valve, 95, in the "aspirate" valve position, 98, and the syringe control valve is in the "fill" valve position, 113, the syringe pump, 100, the syringe plunger, 101, and syringe driver, 102, cause liquid to be aspirated from the sample wells, 104, through the injectors, 83, into tubes, 92.

Injection and chromatographic separation.

When the mechanical transporter, 82, moves the injectors, 83, so that the hollow needles, 84, no longer press against the bottom of a series of wells, 104, the injectors, 83, are no longer connected to the wells, 104. When the pump control valve, 95, is returned to the "pump" position, 97, the flow is resumed from the liquid chromatography pump, 96, through the pump selection valve, 95, the supply tube, 94, union fitting, 93, tubes 92, injectors, 83 and columns, 86. Said flow carries the liquid samples left in tubes 92, and moves it into the columns, 86, thereby injecting the sample into the column. As the flow continues, chromatographic separations take place in the columns, 86. The flow through the columns then traverses the second adaptor fittings, 87, to the detectors, 88, where the passage of various chemical components within the flow are detected. The detectors, 88, are connected to a detector controller, 90, through detector cables, 91.

Other operations.

It will be obvious to those knowledgeable on the state of the art that several auxiliary operations are necessary in liquid chromatography, and that these can easily be performed with the device of Figure 4.

For instance, the tubes 92, injectors 83, and the needles 84 can be rinsed with a rinsing liquid. This is often but not always the same liquid as is used for chromatography. The

mechanical transporter, 83, moves said injectors to a series of wells, used as rinse wells, 105. The needles are pressed against the bottoms of the wells, 105, in the same way as during aspiration. However, instead of aspirating sample, either the liquid chromatography pump or the syringe pump causes rinsing liquid to flow through tubes, 92, injectors, 83, and hollow needles 84, into the rinse wells, 105.

Other manipulations of valves and pumps can be used to rinse aspiration tube 111 and syringe tube, 109.

Series of Injections.

The device of Figure 4, can inject from more than one series of samples wells, 104. For instance, if the mechanical transporter, 82, moves the injectors, 83, to a second set of wells, 106, then the contents of said second set of wells can be chromatographed. In this way, the contents of all, or substantially all of the wells in the microtiter plate, 103, can be chromatographed.

When a microtiter plate with a cell spacing of half or quarter the cell spacing is used, the series of injectors, spaced 9 mm apart, can still be used to chromatographically process all the wells in the microtiter plate. For instance, with a 16-cell row in a 384 well plate, with 4.5 mm spacing, the device of Figure 4 can inject and chromatography samples from wells # 1,3,5 and 7, in the first row. In a second operation, with the mechanical transporter moved by 4.5 mm, the first-row wells # 2,4,6, and 8 can be processed. Then the mechanical transporter moves so that the device is aligned with wells 9, 11, 13, and 15. After a third movement, the wells at # 10,12, 14, and 16 are processed. With a movement in the other axis, all of the wells in the other 23 columns can be processed.

While Figure 4 shows four injectors, 83, it is obvious to those skilled in the art that multiple analyses could easily be performed by another number of injectors, such as two, three, six, eight or twelve, where the number of injectors is an even divisor of the number of rows or of columns in the microtiter plate.

Multiple Rows of Injectors.

While Figure 4 shows the injectors, 83, arranged in a line, it is obvious to those skilled in the art that injectors could be arranged in more than one line.

For instance, FIGURE 6 shows a valuable realization of the invention that has an arrangement of two rows of eight injectors, with the spacing between the rows determined by the spacing of the wells.

There are eight injectors in a first row, 110, with the eight tubes connected into common union fitting, 111. The spacing between the injectors is 9 mm. A second set of injectors is arranged into a second row, 112. The second row, 112, is spaced 27 mm from the first row. which is triple the spacing between wells in the microtiter plate, 103. The sixteen injectors are all mounted to a mechanical transporter, 113. The mechanical transporter, 113, can move the injectors down to press against the wells of the microtiter plate, 103, in order to aspirate a liquid sample into each injector.

Two flow systems, 114, are used, one for the first row, 110, and the other for the second row, 112. It is obvious to those skilled that a single flow system could have been used, or a larger number. For instance, four flow systems could be used, each of which is connected to four injectors.

Alternate way to open the injector valve.

Figure 7 shows a variation in the hollow needle, 84, which is incorporated into an injector, 83. There may be cases where it is not desirable to press a hollow needle, 84, against the bottom of a well, 126. For instance, some samples may contain contamination by solid particles, 125, which it would be disadvantageous to aspirate along with the liquid, 124. In this case, it would be desirable to position the tip of the hollow needle, 84, above the bottom of the well, 126, during aspiration.

In Figure 7, a flat boss, 121, is attached to the hollow needle, 84. The flat boss is attached by means of a threaded collar, 120, which allows the position of the flat boss along the hollow needle, 84, to be adjusted. When the needle is inserted into the well, 126, the flat boss, 121, contacts the upper edge of the well, 126, before said hollow needle contacts the bottom of said well. The pressure of this contact opens the ball valve. One advantage of this variation is to provide for use with samples known to have immiscible liquid phases, 123 and 124, where the aspirating position of the hollow needle, 84, can be adjusted to sample from a particular liquid phase, 124.

A Washing Station.

In Figure 4, some of the wells, 105, in a microtiter plate, 103, were dedicated to being used as a rinsing station for the hollow needles, 84. Figure 8 shows an alternative design, which provides a rinsing station, separate from the wells in the microtiter plate. Two rows of samplers, 110 and 112, are rigidly mounted on a common transporter, 113. The transporter, 113, not only can move the injectors, 110 and 112, to different positions over the microtiter plate, 103, but can also move said injectors to a separate washing station, 131, which has receivers, disposed to permit said injectors to press down on the washing station, 131, and thereby to receive rinsing liquid.

It is obvious to one skilled in the art, that the same arrangement would permit a washing liquid to be aspirated from the washing station, 131.

Control of injection volume.

It is noted in Figure 4, that it is important that sample be aspirated into the tubes, 92, but not into the union fitting, 93 and supply tube 94. Otherwise, when flow is resumed from the liquid chromatography pump, 96, a residual mixture from all the aspirated samples will be introduced into each column, 86. However, if samples are aspirated only part way through tube 92, it may be difficult to control the volume of sample.

In Figure 9, these difficulties are addressed. Figure 9 shows a two-channel system, with columns, 140, attached to an injector block, 141, which contains several injectors, two of which are connected. Sample tubes, 142, are of definite volume, and will determine the volume of the injection. Flow from the LC Pump, 155, goes through the solvent delivery tube, the solvent tee, 146, and the two sample-solvent tubes, 144, to the sample tees, 143. The sample tees, 143, also connect to the syringe pump, 157, through the aspiration tubes, 145, two position valve, 154, valve tubes, 149, syringe tee, 150,syringe tube, 151, syringe valve, 156, and tube, 158.

In this realization, the flow from the LC Pump, 155, always flows during sample aspiration. This requires that the rate of aspiration by the syringe pump, 157, is higher than the flow rate from said LC pump. Unlike the realization of Figure 4, sample is aspirated until the flow of sample over fills the sample tubes, 142, and flows into the aspiration tubes, 145, or even further. After sufficient sample has been aspirated, the injector block, 141, is raised, so that the valves within the injector block close. This ends the aspiration of sample.

The flow from the LC pump, 155, continues, and is directed through the aspiration tubes, 145, ultimately to the syringe valve, 156, where it is either aspirated into the syringe pump, 157, or directed to the vent tube, 152, to vent to waste, 153. This process rinses any remaining sample in the sample tees, 146, aspiration tubes, 145, and other parts of the flow system.

Next, the two-position valve, 148, switches, so that the syringe pump, 157, and syringe valve, 156 are isolated from the aspiration tubes, 145. Now the flow from the LC Pump, 155, has nowhere to go except through the columns 140. So the flow sweeps the sample that resides in the sample tubes into the columns. Note that the volume of each sample is precisely set by the volume of each sample tube, along with a small, defined volume in the injector block. In this way, the two problems of a defined sample volume, and cross-sample contamination are eliminated.

In one realization the following components were used:

Identifier	name	Description
140	column	Thermo Hypersil-Keystone Javelin column, 2.1 mm id, 20mm long, 5-micron packing, coated with Beta Basic C18
141	injector block	--
142	sample tube	8.3 cm long, 0.25 mm id. PEEK
143 sample tee; 146 solvent tee; and 150 syringe tee:		three way, for 1/16" tubing, 10-32 fittings, PEEK
Other tubes: 0.5 mm id, PEEK:		
144	solvent-sample tube	30 cm long
145	aspiration tube	30 cm long
147	solvent delivery tube	25 cm long
149	valve tube	20 cm long
151	syringe tube	30 cm long
158	tube	30 cm long
148	two-position valve	Valco, Cheminert, rotary valve, 6-port
152	vent tube	17 CM 1/16 od pfte
153	vent to waste	--
154	plug	--
155	LC Pump	Pharmacia "Bromma" 2249 Gradient Pump
156 syringe valve and 157 syringe pump:		both part of Cavro XP3000 modular digital pump

Integrated Sample Tubes.

Figure 10 shows another realization of the invention. Here the sample tubes have been incorporated into a sample tube block. In Figure 10, the sample tube block, 168, is rigidly fastened to the injector block, 167, by a fastener, 169. The sample tube block has three sets of interconnected channels lying in a plane. Several cross channels, 176, contain the sample tubes, 175. The sample tubes meet with or penetrate into the injector block. There are sample tube seals, 170, which seal said sample tubes to the injector block, 167, and to the sample tube block, 168. They also seal between the injector block, 167 and the sample tube block, 168.

Each cross channel is intercepted by the solvent channel, 177, which also connects to the solvent-sample tube, 163, which connects to a solvent flow circuit, 162, and thence to an LC Pump, 161. Note that the solvent channel, 177, intercepts the cross channel, 176, between the sample tube seal, 170, and the mixing point, 178, where the flow from the LC Pump, through the solvent channel, 177, meets with the flow to or from the syringe pump, 164, through the aspiration channel, 178.

Each cross channel, 176, is intercepted by the aspiration channel, 178, which also connects to the aspiration tube, 166, which connects to a syringe flow circuit, 165, and thence to the syringe pump, 164. Note that the aspiration channel, 178, intercepts the cross channel, 176, beyond the end of the sample tube, 175, and the mixing point, 178.

With this realization, sample can be aspirated through each sample tube, 175, so that some sample flows past the mixing point, 178, into the aspiration channel, 178, and aspiration tube, 166. The portion of this flow path past the mixing point, 178, can be rinsed by flow from the LC pump 161, solvent-sample tube, 163, solvent channel, 177, and thence, along the outside of the sample tube, 175, to the mixing point, 178.

This realization does not depend on tees for the mixing point, 178, as were used in Figure 9, and permit the sample tube, 175, and mixing point, 178, to be made compactly and precisely, with little dead volume. It also has the advantage that, the volume of the

sample tube, 175, can be adjusted simply by changing the length or inside diameter of said sample tube with no change to other dimensions.

Unequal column flow.

In Figure 10, where a source of flow is split between columns, a possible difficulty is that the flows may not be the same in each column. This difference in flows may cause solvent flows from one cross channel, 176, and sample tube, 177, where the column flow is lower, to flow over to another cross channel, where the column flow is higher.

One way this difficulty can be eliminated or greatly reduced is by arranging, during chromatography, to have some bleed flow out of the sample tube block, 168, into the aspiration tube, 166. For instance, a tube with restricted flow can be mounted in the syringe flow circuit, 165, so that said tube can be connected from the aspirator tube to vent to waste, thereby permitting a low bleed flow to sweep the aspirator channel, 178, and aspirator tube, 166. Alternatively, the bleed flow can flow in the alternative direction. With this arrangement, a difference in flow in a column is accommodated by adjustments in the bleed flow.

Independent Flow sources.

Another realization uses independent flow sources for each column, but still uses a common syringe pump for aspiration. This permits independent control over the chromatographic flow in each channel.

Figure 11 shows a sample tube block, 182, mounted on an injection block, 183, with sample tubes, 184, located in cross channels, 185. Instead of a single solvent-sample tube feeding connecting to multiple cross channels, as in Figure 10, in Figure 11, there are multiple solvent-sample tubes, 187, each of which is joined to a cross channel, 185. There are multiple aspiration tubes, 186, each of which is joined to a cross channel, 185. Seals, 181, are used to seal the various components.

Each solvent-sample tube, 187, is connected to a solvent inlet port, 188, where a source of solvent flow can be connected.

Each aspiration tube, 186, connects to a separate port, 194, on a valve, 190. The valve, 190, in one position, closes off the aspiration tubes, 186. In another position, said valve connects each aspiration tube, 186, to another valve port, 195, which connects to a syringe valve, 191, and syringe pump, 192. For example, the valve, 190, may be a rotary valve, with a rotor with a cross-shaped groove, 196, which can simultaneously connect the separate ports, 194, to another valve port, 195.

This realization permits totally independent control of the chromatographic flow in each column, since independent sources of chromatographic flow are connected to each column, via solvent inlet ports, 188. During aspiration of samples, only a single syringe pump, 192, is needed. Even if the flows in the sample tubes, 184, are not identical during aspiration, this realization permits substantially known and equal injection volumes, since the sample tubes, 184, can be overfilled, and the excess sample can be washed out of the aspiration tubes, 186, by flow of solvent, through the aspiration tubes, 186, and the valve, 190.

I claim:

1. A valve assembly, consisting of a housing, a hollow needle connected to valve ball and seat, said valve ball and seat capable of forming a liquid tight seal for a valve, whereby mechanical pressure on the hollow needle can open the valve thereby opening a liquid flow passage through the hollow needle, past the valve ball and seal, to the interior of the valve assembly, and whereby, in the absence of said mechanical pressure, a spring provides a force that closes the valve and seals said liquid flow passage, even when the pressure within said valve assembly is 350 bars of pressure in excess of the ambient pressure.

2, The method and apparatus of claim 1, whereby the valve assembly is used for opening and closing a liquid flow passage into a liquid flow system used for separation of chemical constituents by chromatography or by electrophoresis,

2. The method and apparatus of Claim one, used for opening and closing a liquid flow passage into a liquid flow system used for introducing a liquid material containing several constituents, so that said constituents can be separated by chromatography or by electrophoresis.

4. The method and apparatus of Claim one, whereby said valve assembly is connected to a pumping system capable of aspirating sample in through the liquid flow passage, by producing a pressure lower than ambient.

5. The method and apparatus of Claim one, where the flow system is in flow communication with a column used for separation by chromatography or by electrophoresis.

6. The method and apparatus of Claim one, where the flow system is rigidly attached to the column.

7. The method and apparatus of Claim one, where the flow system is attached to a tube, of sufficient volume to completely contain the sample of liquid material when it is first introduced.

8. The method and apparatus of Claim one, whereby the valve is opened by causing the tip of the hollow needle to press against a hard surface,

9. The method and apparatus of Claim one, whereby the valve is opened by causing a spacer attached to the side of the hollow needle to press against a hard surface,

10. The method and apparatus of Claim 1, whereby the hollow needle is positioned with its tip in a liquid contained in a container, when the valve is caused to open.

11. A multiplicity of valve assemblies, each consisting of a housing, a hollow needle connected to valve ball and seat, said valve ball and seat capable of forming a liquid tight seal for a valve, whereby mechanical pressure on the hollow needle can open the valve, and whereby, in the absence of said mechanical pressure, a spring provides a force that closes the valve and seals the liquid flow passage, even when the pressure within said valve assembly is 350 bars of pressure in excess of the ambient pressure, wherein said valve assemblies are mounted rigidly together in such a way that said mechanical pressure can be applied to said hollow needles substantially at the same time.

12. The method and apparatus of Claim 11, wherein said valve assemblies are each attached to a tubular column capable of use for separation by chromatography or by electrophoresis.

13. The method and apparatus of Claim 11, wherein said valve assemblies are arranged to take chemical samples from sample containers contained in a fixed rectangular array, wherein the spacing between said valve assemblies is equal or a multiple of the spacing between said containers, and the spacing between said valve assemblies is an integral divisor of the product of the number of said sample containers in a row or column, multiplied by the spacing between said sample containers.

14. A multiplicity of valve assemblies, each consisting of

A housing,

A hollow needle connected to said housing,

Said housing containing a valve capable of sealing a liquid flow passage against 350 bars of pressure,

Said valve assembly connected to a chromatographic column, a source of liquid suitable for driving chemical separations within the chromatographic column, a pumping system

capable of driving said liquid through said chromatographic column, and also capable of aspirating sample in through said needle, when said valve is opened,
Whereby the valve assemblies are rigidly mounted together so that all of the valve assemblies can be moved to locations suited to the aspiration of liquid samples and of rinsing or washing the apparatus.

15. The method and apparatus of Claim 14, wherein said valve assemblies are mounted rigidly together in such a way that said mechanical pressure can be applied to said hollow needles substantially at the same time.

21. I claim an apparatus to make injections of liquid into a chromatographic system, comprising:
An injector with a first internal volume, in contact with a flow from a source of liquid flow into the head of a chromatographic column,
A poppet valve that normally closes off a side passage connecting the first internal volume with a hollow syringe needle,
Said hollow syringe needle being suitable for aspirating a liquid sample from a liquid container, where the hollow syringe needle is sufficiently long to reach the bottom of said liquid container.
A pressurizing means of controlling liquid flow, normally into said first internal volume through a supply tube, and into the chromatographic column,
Said pressurizing means being able to turn off pressure and flow, and to reverse the flow of liquid back into the supply tube, and to control the amount of flow that flows in a reverse direction into the supply tube.
A mechanical mover that is capable of translating the injector, so that the injector can acquire samples that can be transferred to column for chromatographic separation, and can wash or rinse its interior.
Said mover being able to move the injector up to a container of liquid, so that the hollow syringe needle penetrates a passage into the container, and contacts the bottom on said container of liquid,

Said hollow syringe needle is connected to the poppet valve, in such matter that contact of the hollow syringe needle with the bottom of said container of liquid causes said poppet valve to open.

A restoring means which causes the poppet valve to close again, when the hollow syringe needle is no longer in contact with the bottom of the liquid container.

A controlling device, which can cause the other parts to aspirate a pre-determined sample of liquid from the container of liquid and deposit it in a flow of liquid going to the column.

22, a method of making injections of liquid into a chromatographic system, comprising the hardware of claim 21:

Under the control of the controlling device,

Said pressurizing means supplies a flow of liquid suitable for liquid chromatographic separations, and for maintaining the chromatographic system between separations,

For an injection, said pressurizing means interrupts the liquid flow, and lets the liquid pressure drop to a low value,

Said mechanical mover positions the injector adjacent to the container of liquid, so that said hollow syringe needle penetrates a passage into the container, and contacts the bottom of the container, thereby causing the poppet valve to open,

Said pressurizing means reversing the flow of liquid out of the first internal volume of the injector into the supply tube, thereby aspirating a flow of sample from the container of liquid into the injector and then into the supply tube,

Said mechanical mover removes the injector from its position adjacent to the container of liquid, so that said hollow syringe needle no longer is in contact with the bottom of the container, thereby causing the poppet valve to close,

23. A method of cleaning the injection apparatus of claim 21, after an injection is made, Comprising the hardware of claim 1:

Under the control of the controlling device,

While said pressurizing means supplies a flow of liquid suitable for maintaining the chromatographic system between separations,

Said mechanical mover positions the injector adjacent to a second container suitable for receiving liquids, so that said hollow syringe needle penetrates a passage into said second container, and contacts the bottom of the container, thereby causing the poppet valve to open,

Said pressurizing means causing a flow of liquid out of the supply tube, through the first internal volume of the injector, through the hollow syringe needle, into the second container,

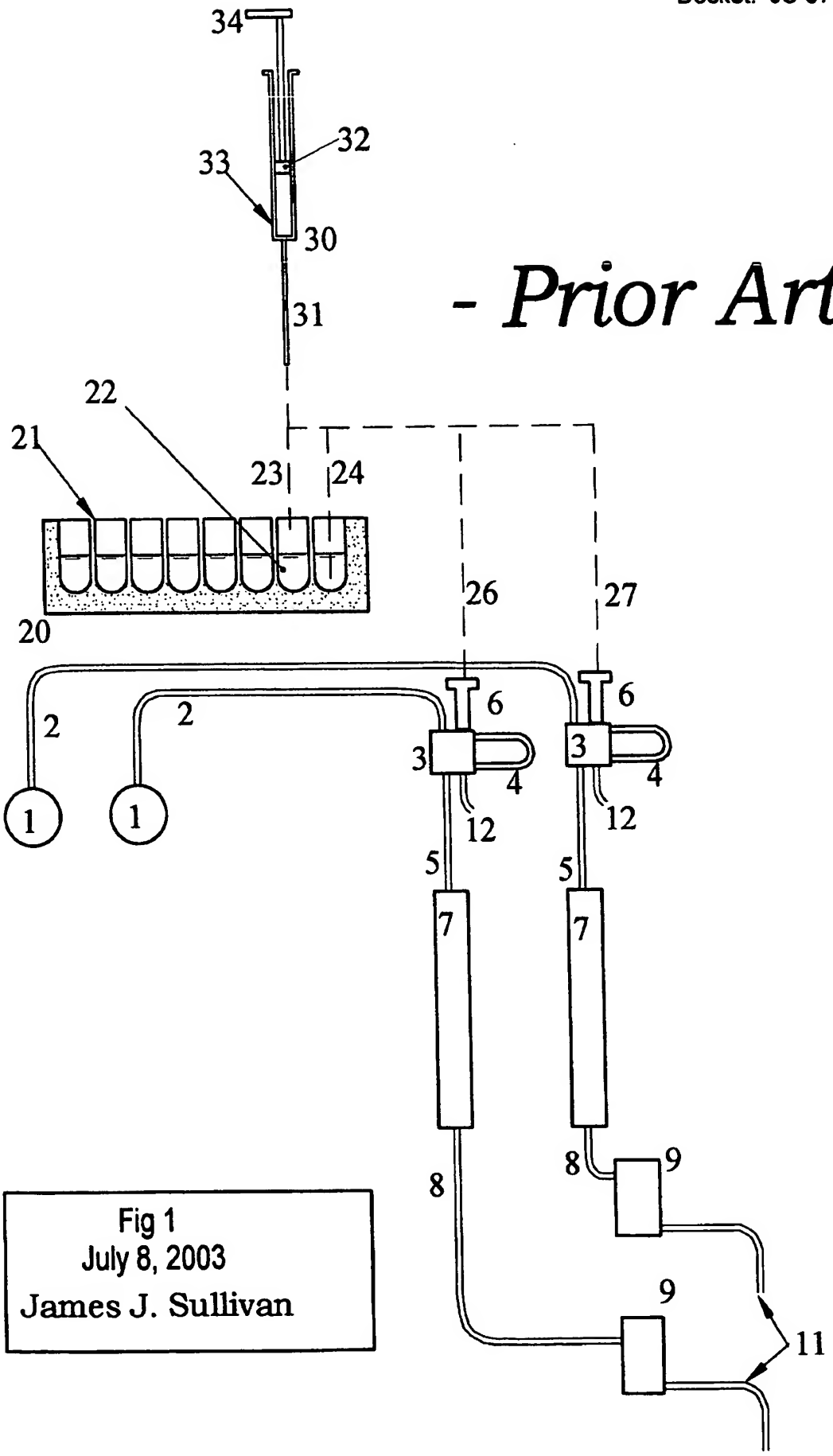
Said controlling device causing the pressurizing means to provide such pressure for such time that substantially all of the residual chemical samples from a previous injection are washed out.

Said mechanical mover removes the injector from its position adjacent to the second container, so that said hollow syringe needle no longer is in contact with the bottom of the container, thereby causing the poppet valve to close,

24. An apparatus suitable for making several simultaneous injections of samples from an array of containers into several chromatographic systems, comprising an array of devices like in claim 1, but fixed together in an array and disposed so that the several needles can penetrate several containers and contact the bottom surface at the same time, where a common moving device is used, a single pressurizing source supplies all of the supply tubes in parallel arrangement, and a single controlling device is used.

25. A method for making simultaneous chromatographic injections using the apparatus of claim 24, and a method similar to claim 22.

26. A method for making cleaning the injection apparatus of claim 4, using a method similar to claim 23.



- *Prior Art*-

Fig 1
July 8, 2003
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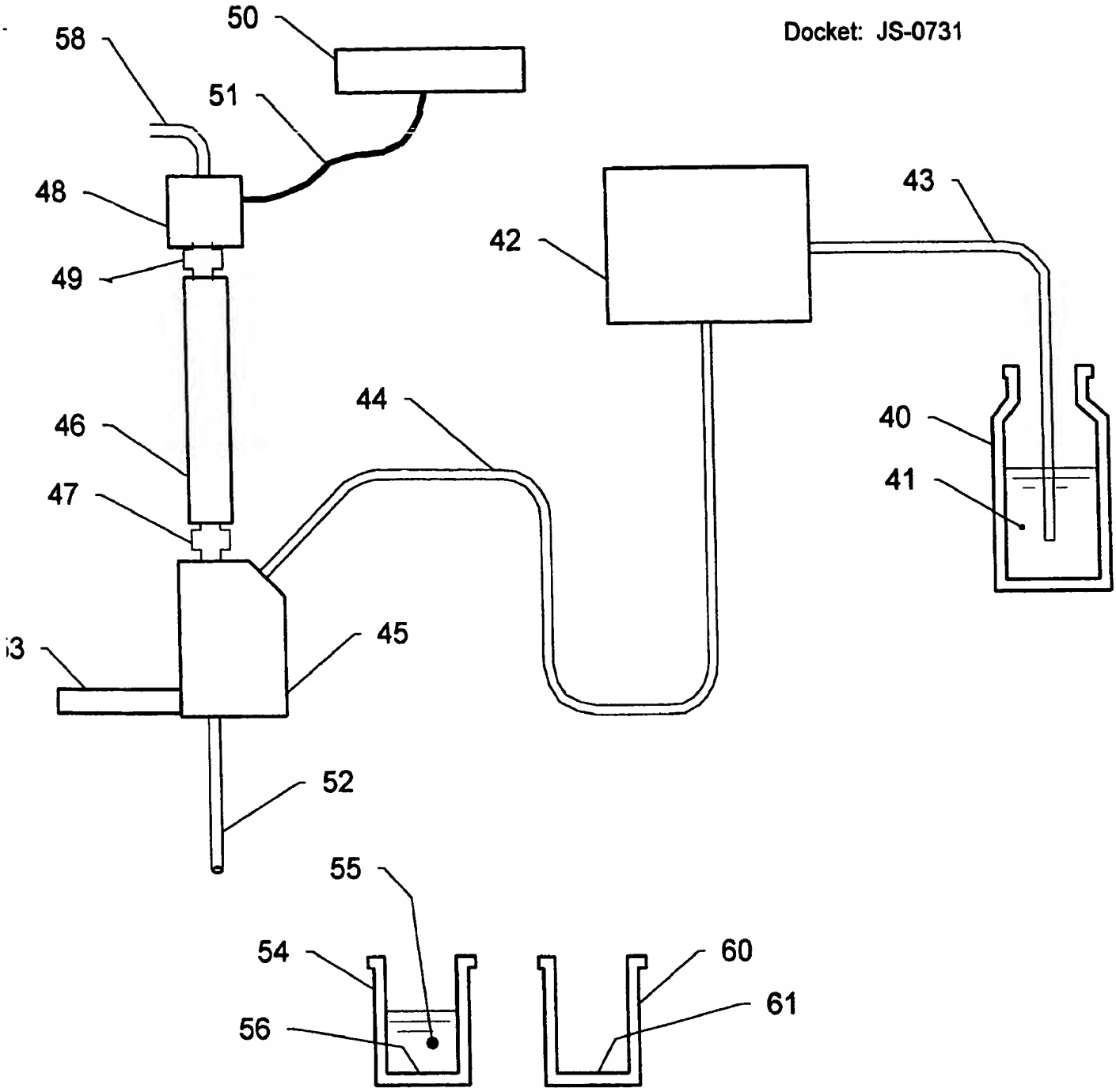

Fig. 2
July 8, 2003

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Docket: JS-0731

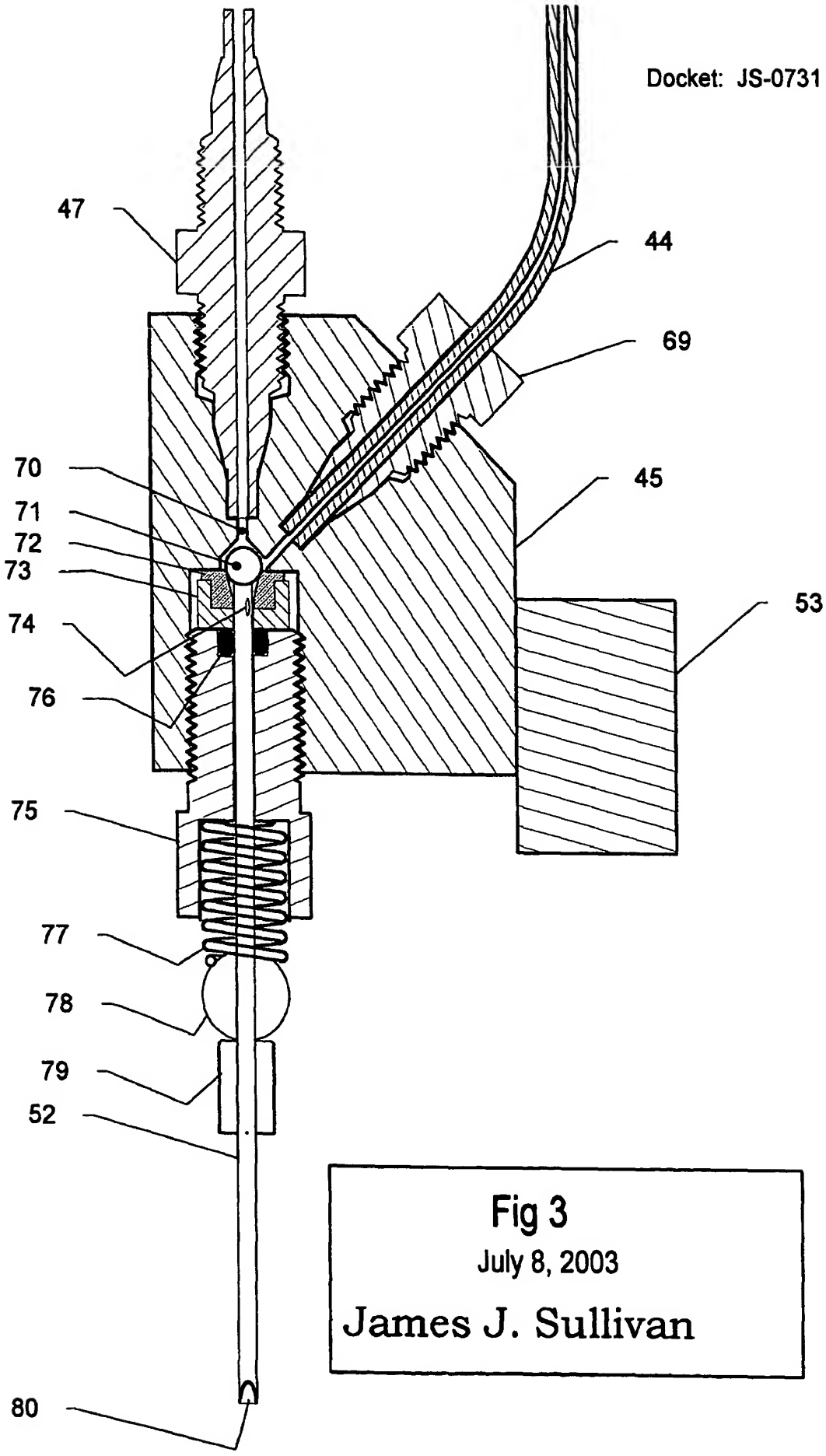

Fig 3

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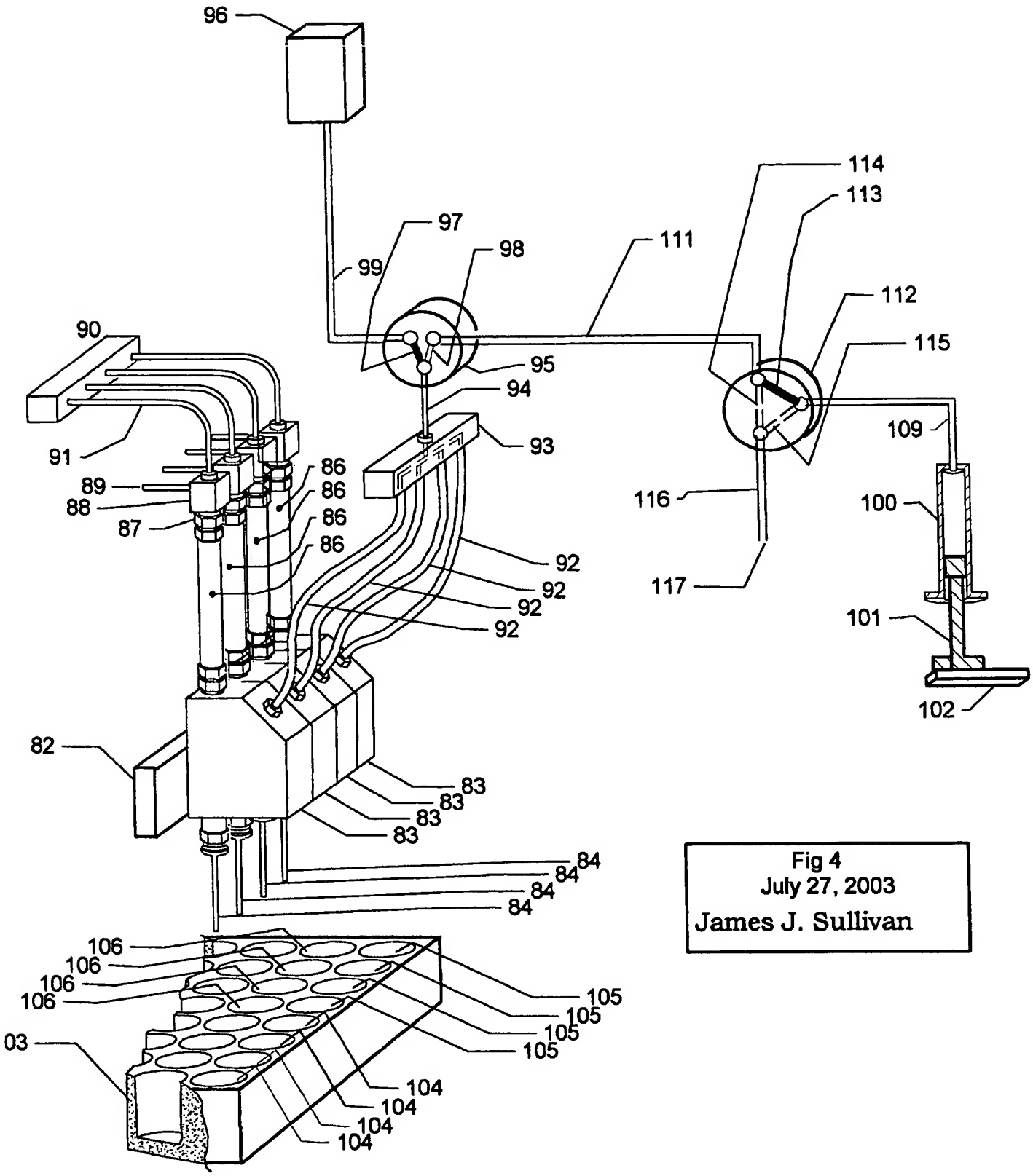

Fig 4
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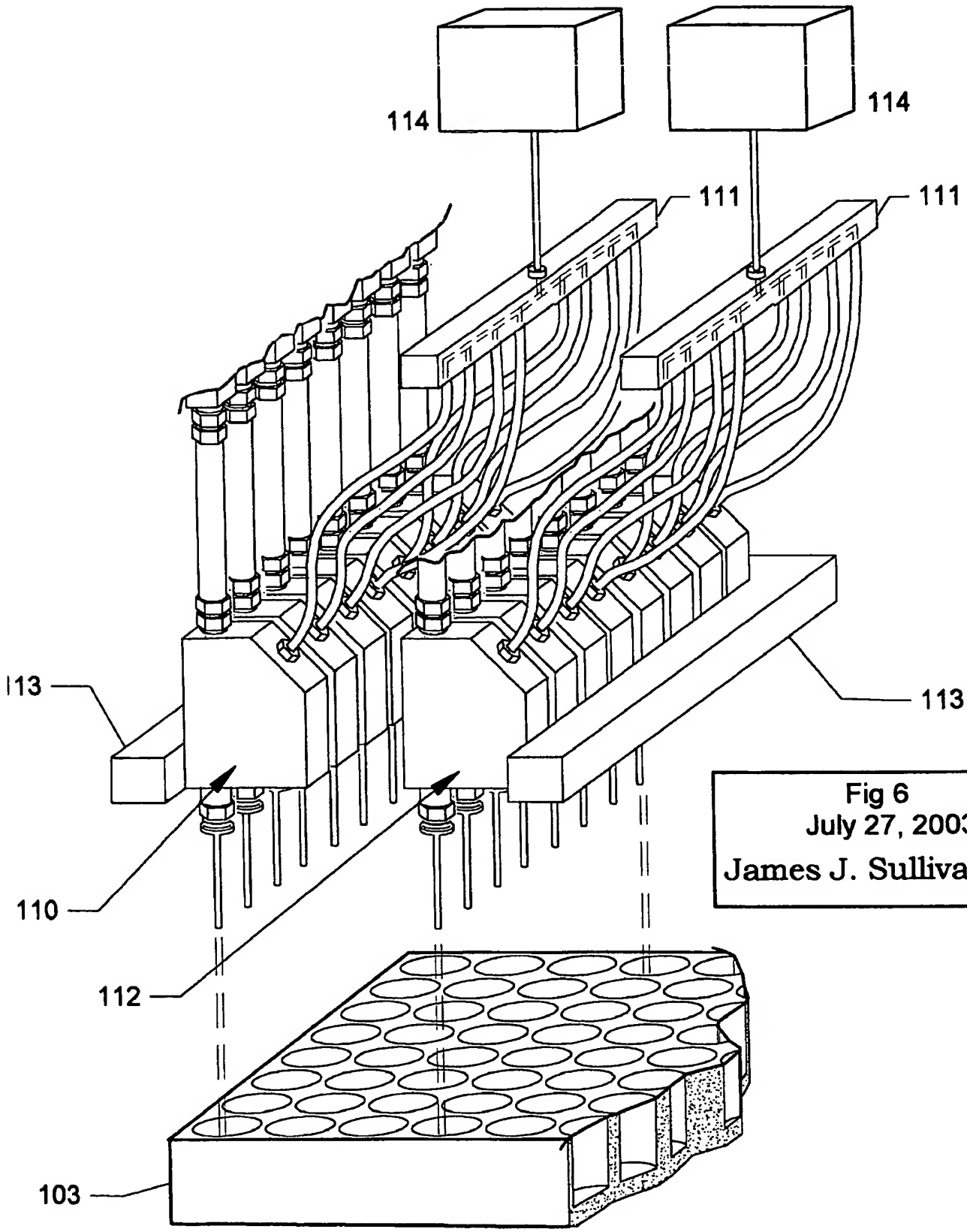

Fig 6
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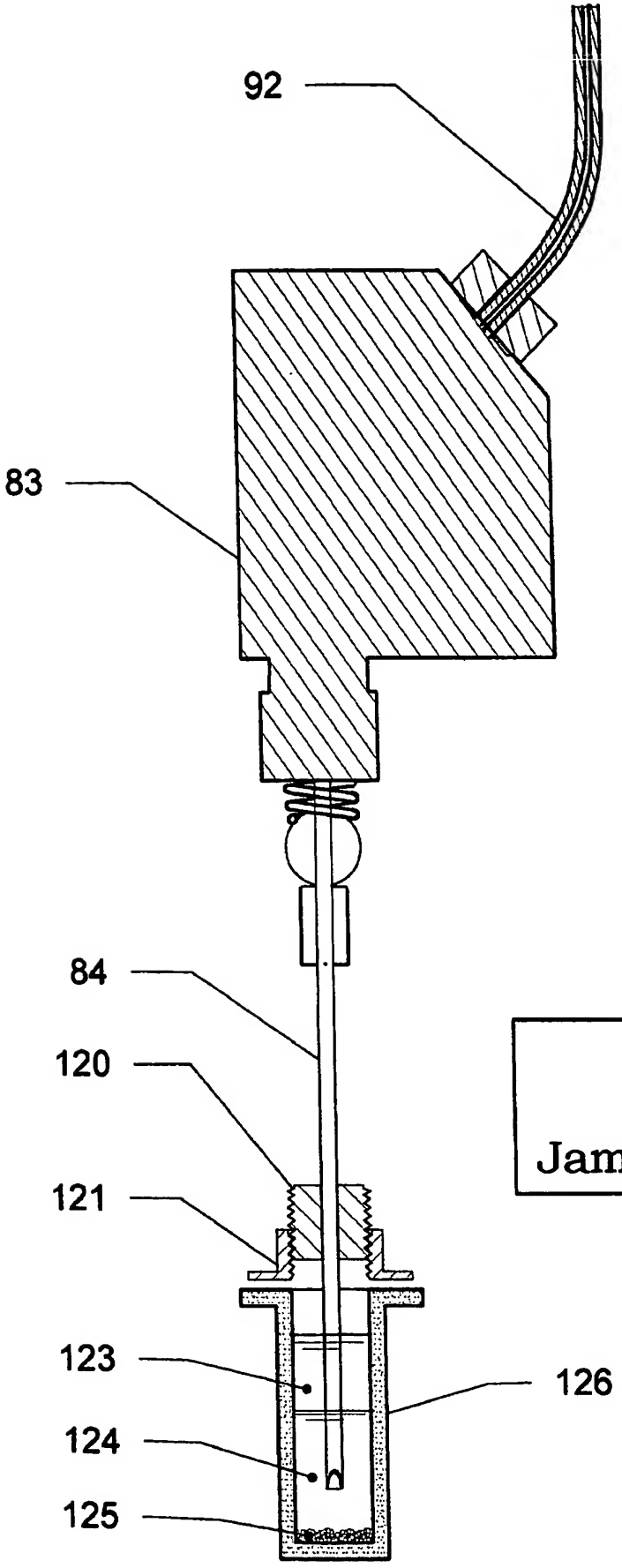

Fig 7
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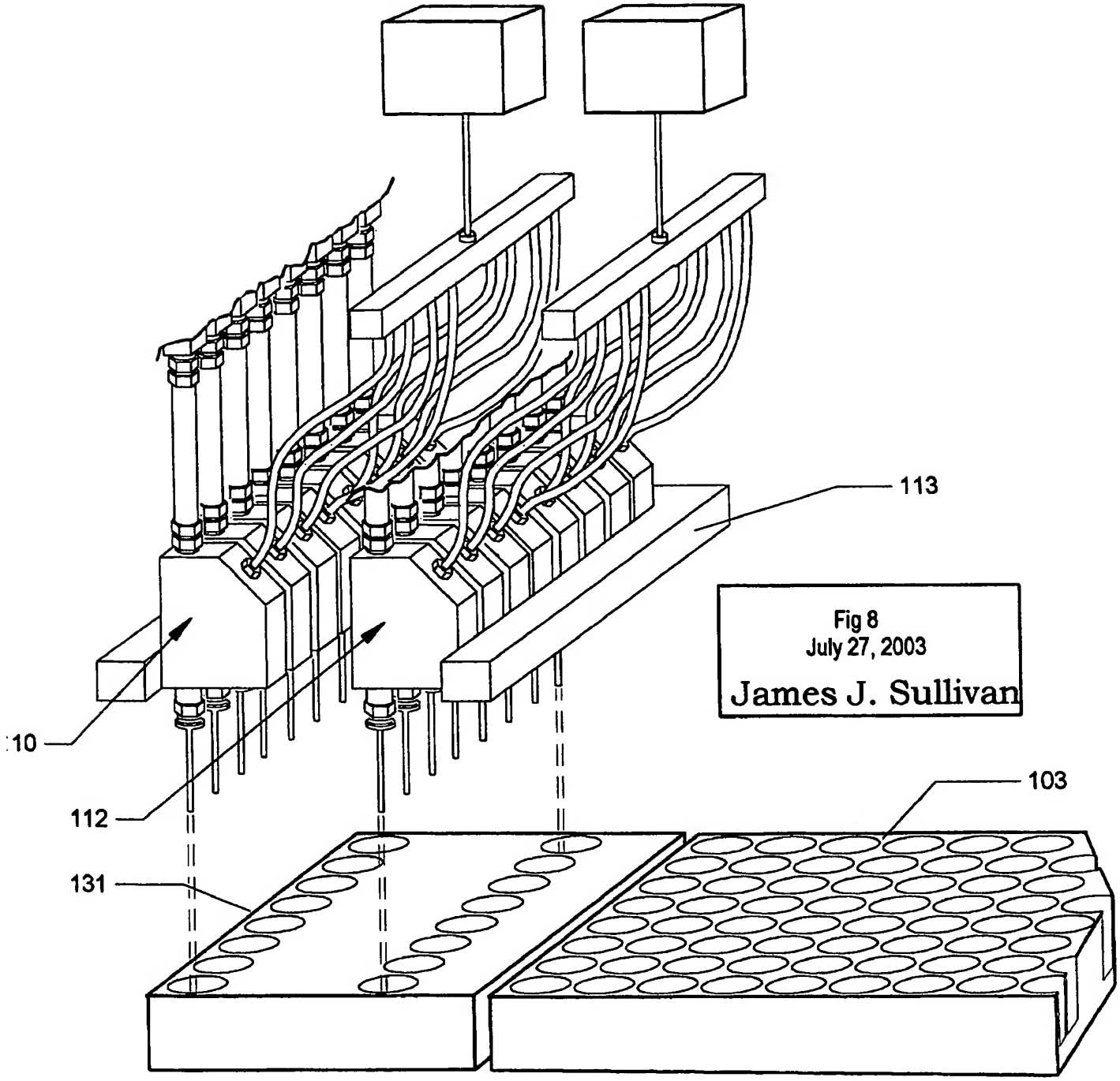

Fig 8
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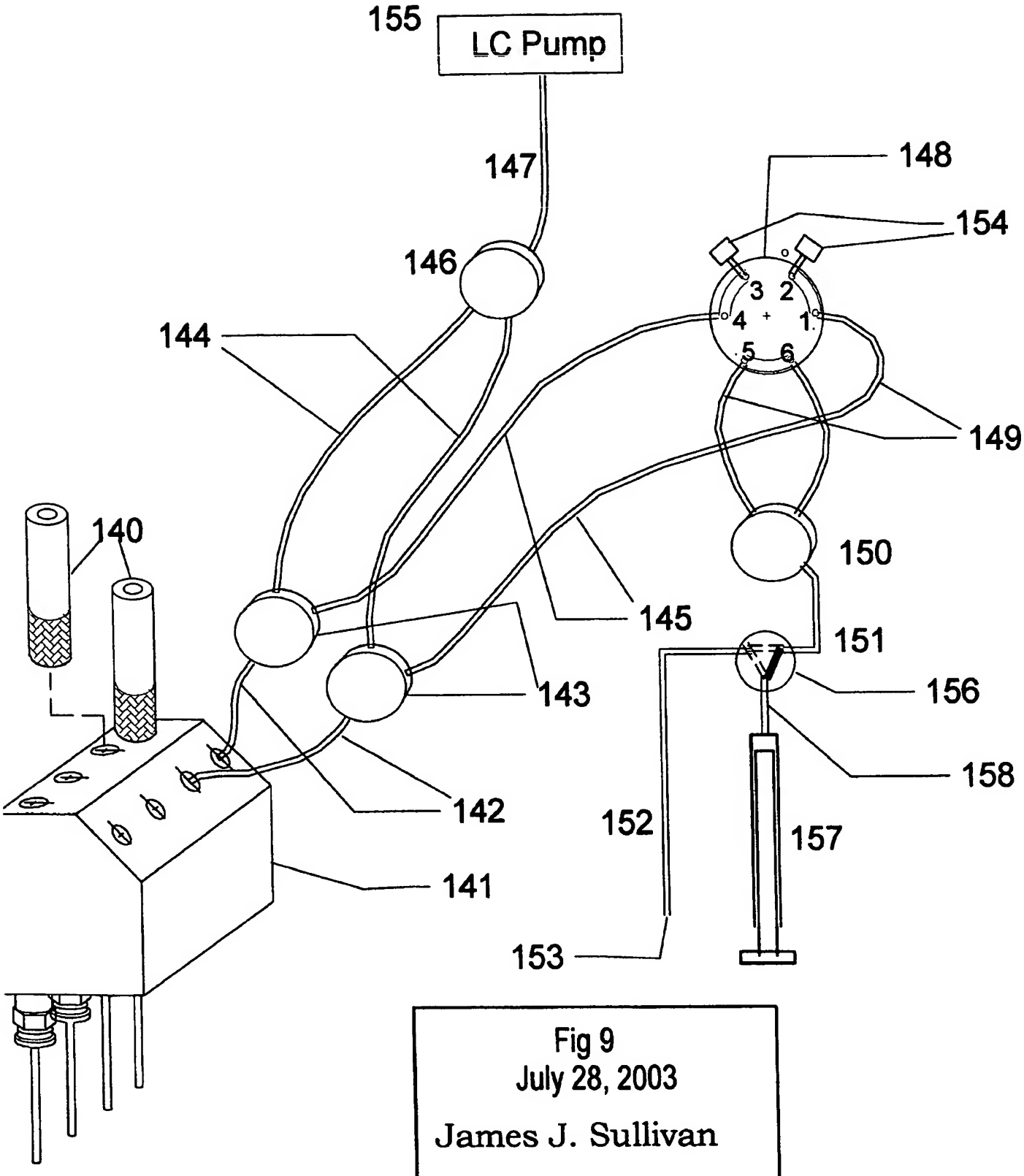

Fig 9
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Docket: JS-0731

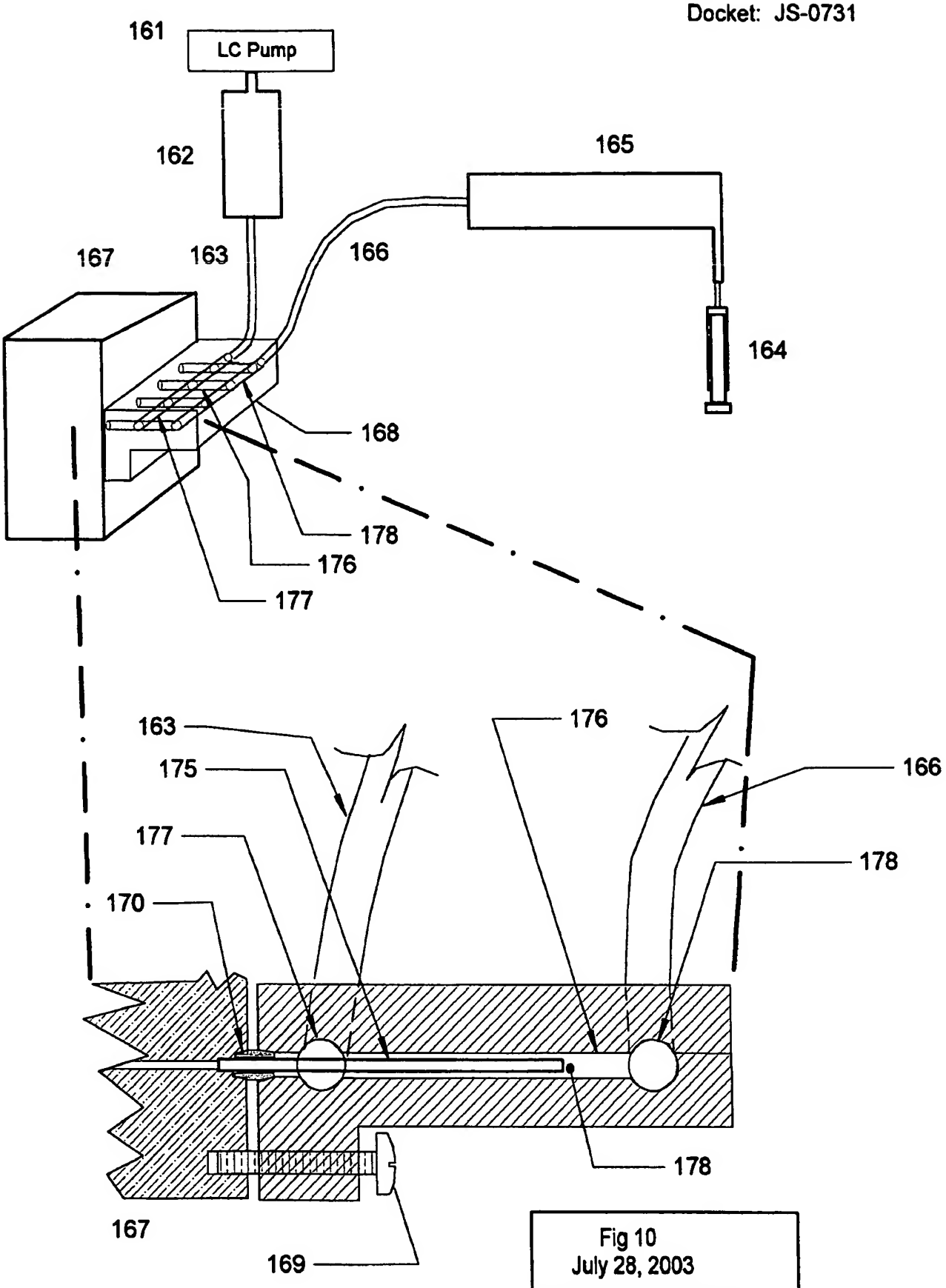

Fig 10
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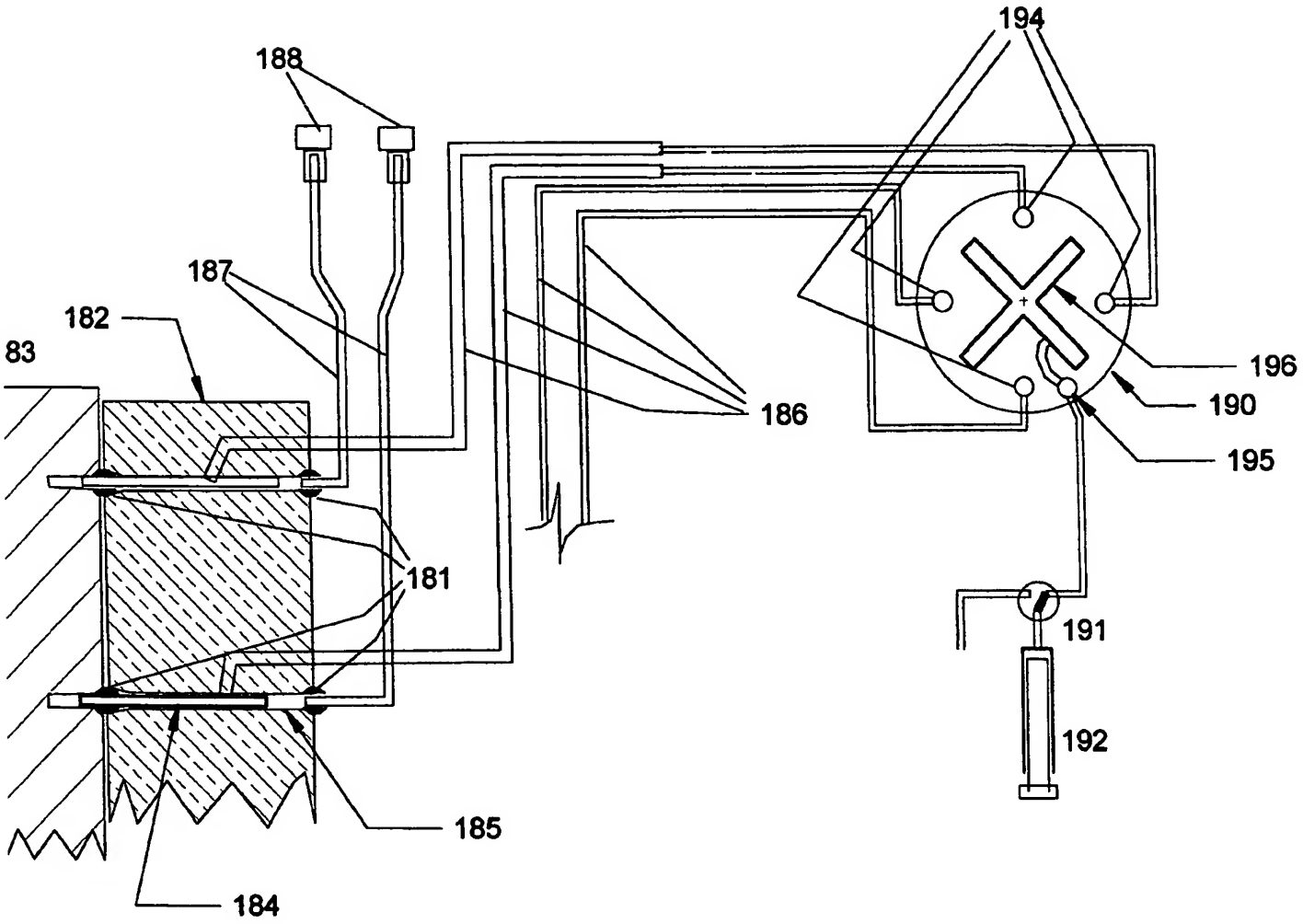

Fig 11
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